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Original Article

Pharmacognostic Studies and *In Vitro* Antioxidant Potential of Traditional Polyherbal Formulation of West Sikkim with *Asparagus* Spp

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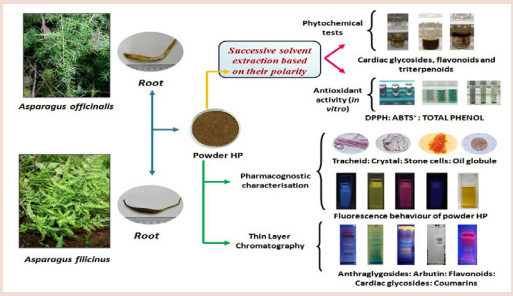
ABSTRACT

Introduction: The powder mixture of the two species of *Asparagaceae* (*Asparagus filicinus* and *Asparagus officinalis*) was found to be used traditionally for the treatment of heart palpitation in west Sikkim. **Objective:** Pharmacognostic characterisation was carried out for the authentication of the powder drug which included powder microscopy, fluorescence analysis and physicochemical characterisation. The presence of any therapeutic potential in HP was also determined by qualitative and quantitative estimation of phytochemicals along with free radical scavenging activity of various successive solvent extracts (based on their polarity). Thin layer chromatography (TLC) of the powdered HP was also done. The standard software SPSS (ver. 15.0) and XLSTAT 2009 (Addinsoft) and Smith's Statistical Package were used for different statistical analysis. **Results:** Powder microscopy of HP revealed the presence of calcium oxalate crystal, tracheids, stone cells etc. Various fluorescence colours were exhibited by HP on UV after reacting with different chemical reagents. The analysis values were also obtained in a satisfactory way. TLC and qualitative phytochemical analysis revealed the presence of some active phytoconstituents. Among all the solvent extracts, acetone, heptane, ethyl acetate and benzene extracts showed higher antioxidant potential. **Conclusion:** The results support the use of HP as a traditional medicine and further purification should be done for the identification of bioactive phytoconstituents responsible for its antioxidant activity.

Key words: Antioxidant, Pharmacognostic evaluation, Phytoconstituents, Successive solvent extraction, Thin layer Chromatography.

SUMMARY

- HP powder prepared from the roots of *Asparagus officinalis* and *Asparagus filicinus* is used to cure heart palpitation in the traditional system at a village in west Sikkim.
- Powder was subjected for some pharamacognostic characterisation methods and the result of which might be helpful in authentication of the traditional drug.
- Some phytoconstituents such as anthraglycosides, arbutin, cardiac glycosides, flavonoids, bitter principles and coumarins were revealed on the sample by separating through thin layer chromatography. Alkaloids and saponins were absent.
- Qualitative evaluation of phytochemicals revealed presence of cardiac glycosides and flavonoids in all solvent extracts while amino acid and triterpenoids in few extracts only and showed absence of tannin, resin and alkaloids.
- Antioxidants potential varied in different solvent extracts but overall acetone and heptane extracts were better than other extracts.



PICTORIAL ABSTRACT

Abbreviations used: HP: powder polyherb for heart palpitation, HPHx: Hexane extract, HPHp: Heptane extract, HPBz: Benzene extract, HPEa: Ethyl acetate extract, HPCl: Chloroform extract, HPAc: Acetone extract, HPBu: Butanol extract, HPEt: Ethanol extract, HPMt: Methanol extract, HPAq: Aqueous extract, TLC: Thin layer chromatography, DPPH: 1, 1-diphenyl-2-picrylhydrazyl scavenging activity, ABTS+: 2,2'-azinobis-3-ethylbenzothiozoline-6-sulphonic acid scavenging activity, SO: Superoxide scavenging activity, NO: Nitric oxide scavenging activity, MC: Metal chelating activity, FRAP: Ferric reducing antioxidant power, TPC: Total phenol content, TFC: Total flavonol content, TOPC: Total orthodihydric phenol content, GAE/mg EW: Gallic acid equivalent/mg extractive weight, QE/mg EW: quercetin equivalent/mg extractive weight, AAE/mg EW: Ascorbic acid equivalent/mg extractive weight.

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INTRODUCTION

Since ancient times, plants have been used as herbal medicines to heal various ailments. Recently, there is widespread interest growing on drugs derived from plants because they are reliable, safe and cost effective compared with expensive synthetic drugs causing adverse effects in human health. Most of the noble drugs used in modern medicines are produced indirectly from medicinal plants[1] and about 90% of raw materials come from wild sources. But ethnomedicinal knowledge has still now confined to certain areas or group of people due to the lack of effective scientific communication.[2] Hence it is quite essential to establish the potential of herbal medicine and encourage the study of folk knowledge about medicinal plants.[3] In fact pharmaceutical and clinical investigations have raised the position of medicinal plants by identifying the active principle components present in them and elaborating their role in human and animal systems.[4]

Sikkim is a small Himalayan state (7096 km^2 geographical area) in Northeast region of India harbouring an extremely rich diversity of medicinal plants. Over the ages, Sikkim has developed rich cultural practices of folk medicines.[5] *Asparagus officinalis* Linn. and *Asparagus filicinus* Buchanon-Hamilton ex. D.Don., both belonging to family *Asparagaceae* are commonly used ethnomedicinal plants. Young stems of *A. officinalis* are used for the treatment of constipation and intestinal disorders.[6] Tubers of *A. filicinus* is traditionally used for the treatment of diarrhoea, dysentery,

diabetes[7] and it was also found to have antiviral activity against Influenza virus A.[8] Our survey revealed that the polyherbal formulation (HP) is made by mixing the roots of both the plants by an herbal practitioner for the treatment of heart palpitation. However the medicine prepared by traditional methods may not have the desired quality and batch to batch consistency. Hence this formulation required standardization with scientific parameters including organoleptic characters, physicochemical analysis and microscopy.

Recently the role of medicinal plants in disease control or prevention has been credited to antioxidant properties of their constituents usually polyphenolic compounds.[9] The intake of natural antioxidants are related to minimizing the risks of common but hazardous diseases like cancer, diabetes, cardiovascular problems, and many other diseases related with ageing.[10,11] Natural antioxidants have been observed in various unexplored plant resources like wild fruits,[12] leafy vegetables[13] and even bryophytes.[14]

Extraction of antioxidants from a plant material can be done by using different techniques and different solvents as these compounds have diverse chemical nature and often unique distribution in the plant matrix.[15,16] Solvent extraction is the most commonly used method for recovery of the plant antioxidants; however, the yields and antioxidant value of the plant extracts are strongly dependent on the polarity of the solvent plus the chemical nature of the isolated compounds.[17]

Literature survey did not yield any information about pharmacognostic studies of the above polyherb. There were no records provided on the antioxidant activity also. Therefore this study was focused on the standardization of HP, which was made from the roots of *A. officinalis* and *A. filicinus*, by measuring various attributes such as powder microscopy, fluorescence analysis and physicochemical studies. To explore the presence of active phytoconstituents, qualitative and quantitative phytochemicals content were also done along with free radicals scavenging activities of HP extracted in different solvents.

MATERIALS AND METHODS

Collection and authentication of plant material

A polyherbal powder sample prepared from the roots of *A. officinalis* and *A. filicinus* in equal ratio was collected from an herbal practitioner from Uttarey, West Sikkim in April, 2014. The herbarium specimen was identified and authenticated by Dr. A.P. Das, Professor, Taxonomy and Environmental Biology Laboratory, Department of Botany, University of North Bengal. The voucher specimens were deposited at laboratory herbarium, Taxonomy and Environmental Biology Laboratory, University of North Bengal against accession no. 09724/Tag no. E.S. 05 and 09723/Tag no. E.S. 04 for *Asparagus filicinus* Buchanon-Hamilton ex. D.Don., and *Asparagus officinalis* Linn. respectively.

Sample preparation

The powder sample was extracted through soxhlet apparatus in 10 different solvents based on their polarity. The solvent extracts were hexane (HPHx), heptane (HPHp), benzene (HPBz), ethyl acetate (HPEa), chloroform (HPCl), acetone (HPAc), butanol (HPBu), ethanol (HPEt), methanol (HPMt) and water (HPAq). The samples were then evaporated and reconstituted in methanol. These extracted samples were used for qualitative and quantitative estimation of phytochemicals and determination of free radical scavenging activities. For TLC and pharmacognostic studies, the sample was used in its powder form.

Pharmacognostic characterization

Organoleptic evaluation and Powder microscopy

The colour, odour, taste and texture of the powder of HP were observed and recorded.[18] Microscopic examination was carried out in a standard method.[19] A small amount of powder of HP was taken on glass slide and mounted on glycerine and observed under microscope. For the observation of lignified tissues, powder was stained with alcoholic solution of phloroglucinol followed by concentrated HCl. Similarly the powder was also stained with N/10 iodine solution to observe the starch granules and for the identification of fixed oil and fats, Sudan III were used.

Fluorescence behaviour

The powder sample as such and after treatment with various chemical reagents was subjected to fluorescence analysis. Observations were made under visible light and under UV light of short (254 nm) and long wave length (365 nm) separately.[20]

Physico-chemical parameters

Coarse powder of the plant root was used to perform quality control parameters such as total ash, acid insoluble and water soluble ash, water and alcohol soluble extractive values and loss on drying.[21] Three determinations were carried out for each parameter.

Preliminary photochemical studies

The HP powder extracted in various solvents were subjected for preliminary phytochemical screening to observe the presence or absence of phytoconstituents like tannin, triterpeniods, amino acid, resin, cardiac glycosides and flavonoids by the standard methods.[22-25]

Thin layer chromatography

TLC was performed to analyse the variation in bioactive chemical constituents.[26] Readymade TLC plates (coated with silica gel 60 F_{254} on aluminium sheets) purchased from Merck Germany were used. The powdered sample was extracted with different procedures for the identification of each of the active constituents i.e. anthraglycosides, arbutin, cardiac glycosides, flavonoids, bitter principles, saponins, coumarins and alkaloids. The mobile phase solvent systems used were ethyl acetate: methanol: water (100:13.5:10) for the detection of anthraglycosides, arbutin, cardiac glycosides, bitter principles and alkaloids. The mobile phase ethyl acetate : formic acid : glacial acetic acid : water (100:11:11:26) was used for flavonoids identification and for the identification of saponin, the solvent system of chloroform: glacial acetic acid : methanol: water (64:32:12:8) was used while for the identification of coumarins, toluene : ethyl acetate (93:7) was used. The developed chromatograms were analyzed for presence of drug constituents by spraying with an appropriate group reagent/s. The chromatograms were then observed under UV-254 nm and UV-365 nm light. Photos were taken with DSLR Nikon camera (D-3200) and the R_f values were calculated with the following formula.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Quantitative evaluation of phytochemicals

Total phenol content (TPC)

Folin-Ciocalteu reagent was used to determine the TPC of HP.[27] The absorbance was measured at 725 nm in UV-visible spectrophotometer. Total phenol values were expressed in terms of Gallic acid equivalent (GAE)/mg Extractive weight (EW).

Total flavonol content (TFC)

TFC was measured with the mixture consisted of methanolic extract with Sodium nitrite ($NaNO_2$), Aluminium chloride ($AlCl_3$) and Sodium hydroxide (NaOH).[17] The absorbance was measured at 510 nm. TFC in different extracts was calculated as quercetin equivalent (QE)/mg EW.

Total ortho-dihydric phenol content (TOPC)

TOPC was estimated according to standard method by using Arnow's reagent.[28] The OD values were taken at 515 nm. TOPC was calculated from a catechol standard curve.

Evaluation of antioxidants

DPPH radical scavenging activity

The free radical scavenging activity of all the solvent fractions of HP was evaluated using DPPH.[29] Discoloration of these extracts was measured at 517 nm after incubation for 10 minutes in dark at room temperature. Percentage of inhibition was calculated with the following formula:

$$\frac{I=A_0-A_1}{A_0} \times 100 \qquad \text{Equation (1)}$$

where A_0=absorbance of the control, A_1=absorbance of the sample (different fractions of HP), I=percent inhibition of DPPH by the sample.

ABTS⁺ scavenging activity

The ABTS⁺ free radical scavenging activity of all the solvent fractions of HP was done.[30] The diluted ABTS⁺ solution (2 ml) was added to 1 ml methanolic sample. The scavenging activity of sample was observed and absorbance was read at 734 nm after 10 minutes of incubation. The percentage inhibition was calculated by using Equation (1).

Superoxide scavenging assay (SO)

A standard method was used with some slight modifications to carry out SO scavenging activity.[31] Superoxide anions are produced in the PMS/NADH-NBT system. The decrease in absorbance shows the radical scavenging activity of the sample which was measured at 560 nm. The inhibition percentage was calculated by Equation (1).

Inhibition of nitric oxide production (NO)

At a physiological pH, aqueous sodium nitroprusside generates nitric oxide (NO) which produces nitrite after interacting with oxygen which can be estimated by Greiss reagent.[32] The diazotization of nitrite with sulphanilamide following its coupling with naphthylethylenediamine forms chromophore, the absorbance of which was measured at 546 nm. Equation (1) was used to calculate the percentage inhibition.

Metal chelating assay (MC)

The chelation of ferrous ions by HP extracts was estimated.[33] The colour complex formed by the chelation of Fe^{2+} by ferrozine was reduced by the chelating activity of sample competing with ferrozine was measured at 562 nm. The inhibition percentage was calculated using Equation (1).

Ferric reducing antioxidant power (FRAP)

The FRAP of the HP extracts was determined on the ability of the sample to reduce ferric ions.[34] Absorbance was measured in UV-VIS spectrophotometer at 700 nm. Ascorbic acid was taken as standard.

Statistical analysis

The standard software SPSS (ver. 15.0) was used for different statistical analysis. One-way analysis of variance (ANOVA) was used to compare the differences and the means which were considered significant at $p \leq 0.05$. Correlation and regression analysis were done by using XLSTAT 2009 (Addinsoft) and Smith's Statistical Package.

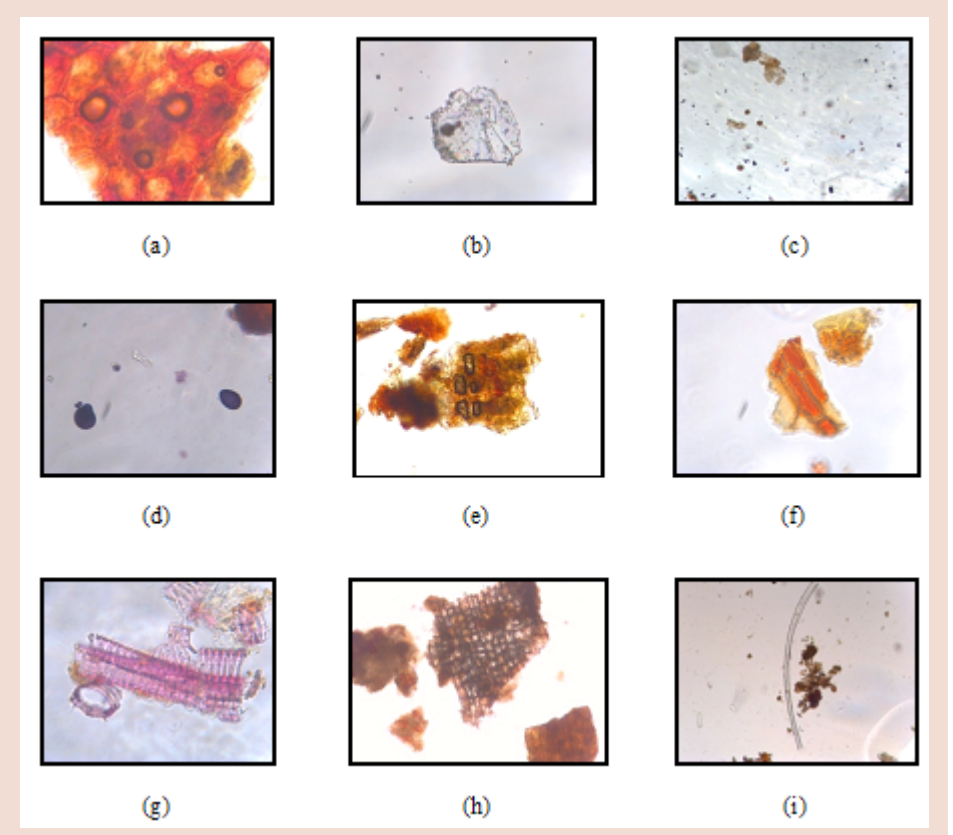


Figure 1: Powder microscopy of polyherb, HP

a) Collenchyma b) Prismatic calcium oxalate crystal c) Oil globules d) Starch e) Stone cells f) Parenchymatous cells g) Scalariform tracheid h) Cork cells i) Fiber

Table 1: Fluorescence analysis of powder HP

Reagents	Visible	UV-254	UV-365
Powder + distilled water	Green beige	Azure blue	Brilliant blue
Powder + 50% KOH	Red orange	Colourless	Black green
Powder + benzene	Sulphur yellow	Curry yellow	Broom yellow
Powder + 50% benzene	Fern green	Colourless	Raspberry red
Powder + chloroform	Colourless	Colourless	Distant blue
Powder + 50% chloroform	Fern green	Pearl blackberry	Rose
Powder + nitric acid (conc.)	Broom yellow	Colourless	Colourless
Powder + 50% nitric acid	Broom yellow	Colourless	Colourless
Powder + 10% ferric chloride	Broom yellow	Colourless	Colourless
Powder + methanol	Colourless	Cobalt blue	Ultramine blue
Powder + 50% methanol	Khaki grey	Grey blue	Gentian blue
Powder + ethanol	Colourless	Cobalt blue	Ultramine blue
Powder + 50% ethanol	Olive grey	Pearl night blue	Traffic blue
Powder + glacial acetic acid (conc.)	Reed green	Yellow olive	Brown beige
Powder + 50% glacial acetic acid	Green beige	No colour	Ultramine blue
Powder + sulphuric acid (conc.)	Ochre brown	Colourless	Blue grey
Powder + 50% sulphuric acid	Ochre yellow	Colourless	Grey blue

RESULTS

Organoleptic and powder microscopic evaluation

The powder of HP was brown in colour, slightly rough in texture and slightly bitter in taste. It had a characteristic smell. Powder microscopy revealed calcium oxalate crystal, parenchymatous cells, fiber, collenchyma, stone cells, cork cells and scalariform tracheid (Figure 1).

Fluorescence analysis

The powder of HP exhibited different colours after treating with different chemical reagents. The colours were observed in daylight and under ultraviolet (UV) light at 254 nm and 365 nm. The colours were identified and noted down in Table 1 using the standard colour chart of RAL.

Physicochemical characteristics

The results obtained from physicochemical studies were given in Table 2. The result of total ash (20.24%) showed that the powder HP contains both organic and inorganic matter. Total ash is mainly essential to check the purity of drugs showing the presence or absence of metallic salts or silica[35,36] which was found to be 20% w/w. The percentage of ash was calculated with reference to the air-dried powder. The acid insoluble ash was noted as 16% w/w while the presence of material exhausted by water i.e. the water soluble ash was recorded to be 14% w/w. Determination of extractive values is another way of checking the purity of the herbal formulation. The water soluble extractive value was found to be 3.21% w/w which signifies that more amount of constituents in HP was soluble in water than in alcohol (0.441% w/w). The percentage of loss on drying i.e. the moisture content in the powder was found to be 10.52% w/w.

Table 2: Physicochemical analysis of powder of HP

Parameter	Value
Total Ash(%) w/w	20.243
Acid insoluble ash(%) w/w	16.011
Water soluble ash(%) w/w	14.01
Alcohol soluble extractive value(%) w/w	0.441
Water soluble extractive value(%) w/w	3.211
Loss on drying(%) w/w	10.52
pH (1% w/v)	6.79
pH (10% w/v)	6.38

Thin layer chromatography

Preliminary investigation of active constituents in plants was carried out by thin layer chromatography (TLC) technique. After applying specific spraying reagents for a particular active constituent, the results of TLC showed the presence of anthraglycosides, arbutin, flavonoids, cardiac glycosides and coumarins in HP. R_f values were calculated for all the spots and the results of this analysis are described in Table 3.

Qualitative phytochemical analysis

The pharmacological activity of the crude drug mainly depends on the metabolites present in it. Thus preliminary screening of phytochemicals which was performed to establish a chemical profile of a crude drug was a part of chemical evaluation.[37,38] These phytochemical tests (Table 4) revealed the presence of cardiac glycosides and flavonoids in different extracts of HP obtained by using different solvents. Tannins, alkaloids and resins were absent while amino acid was found to be present in aqueous extract only and triterpenoids were found to be present in few extracts (acetone, butanol, ethanol, methanol and water).

Antioxidant activity

In the present study, various methods such as DPPH, $ABTS^+$, SO, NO, MC, FRAP were used to evaluate the antioxidant potential of HP. The sample was prepared in a soxhlet apparatus successively in various solvents (non-polar to polar). TPC, TFC and TOPC were also estimated quantitatively for HP (Table 5). The highest TPC was exhibited by heptane extract (361.41 mg GAE/mg EW) followed by acetone (272.99 mg GAE/mg EW). The highest TFC content (15.45 mg GAE/mg EW) was shown by benzene extract. For the radical scavenging activity, it is very clear from Table 5 that the acetone extract of HP showed the lowest IC_{50} values for DPPH, $ABTS^+$ and superoxide scavenging assays along with highest ferric reducing power indicating the highest antioxidant activity. The same extract showed excellent quantity of TPC and TFC also.

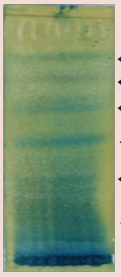
DISCUSSION

Traditional medicine has always played an important role in primary health care. But for the proper utilization of this medicine, each and every formulation mentioned in our indigenous knowledge of medicine should be scientifically evaluated.[39] Therefore the powder of HP was subjected to certain standardization parameters. Despite of the availability of various modern tools for the evaluation of plant drugs, powder microscopy is still considered as the simplest and cheapest method for identification of the source materials.[40] When powder of HP was subjected to microscopic analysis, starch granules were revealed after staining with iodine solution while Sudan III stain showed the presence of

Table 3: Detection of active phytoconstituents in powder HP by TLC method

Ethyl acetate: methanol: water (100:13.5:10)

Red band=Anthraglycosides

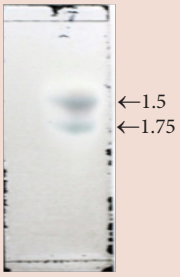


Blue band=Arbutin

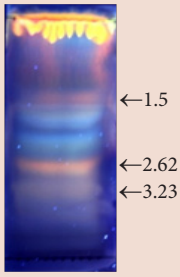
Ethyl acetate: Formic acid: glacial acetic acid: water (100:11:11:26)

Red band=Bitter Principles

Toluene: Ethyl acetate (93:7)

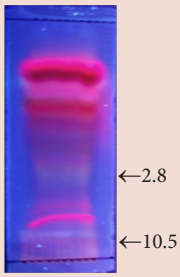


Blue band=Cardiac Glycosides



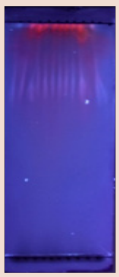
Yellow to orange bands=Flavonoids

Chloroform: Glacial acetic acid: Methanol: Water (64:32:12:8)



Blue band=Coumarin

Ethyl acetate: methanol: water (100:13.5:10)



Violet band=Saponins (Absent)

Brown band= alkaloids (Absent)

oil globules in the powder. Lignified tissues appeared pink in colour distributed abundantly after staining with phloroglucinol solution. Fluorescence study is also an important parameter for standardization of crude drug. Various chemical constituents present in plant materials exhibited fluorescence when suitably illuminated. Fluorescence colour of each compound is very specific. But if those chemicals itself are not fluorescent then they may be treated with different chemical reagents to attain fluorescence.[41] The quality and purity of crude drugs can also be checked by ash values. It indicates the presence of various impurities in crude drugs due to adulteration or incorrect processing during drying or storage or formulation. The water soluble ash shows the amount of acid, sugars and inorganic matter present in the powder while acid insoluble ash shows mainly silica thus indicating the contamination with earthy materials.[42] The extractive values are useful to evaluate the chemical constituents present in crude drug and also help for estimation of specific constituents soluble in a particular solvent. For any drugs and herbal formulation, low moisture content is essential for higher stability of drugs. The general requirement for moisture in a crude drug should not be more than 14%.[43] Excess moisture content may support the growth of fungi and may cause contamination by other microorganisms resulting into the degradation of drug. But HP powder showed 10% of moisture content i.e. loss on drying percentage (Table 2) which is not too high to encourage the growth of microorganisms. The pharmacological importance of a drug is attributed to the various secondary metabolites present in it and a particular compound might possess a clinical significance. Therefore it is essential to separate the compounds present in the plants with an appropriate chromatographic method. TLC technique has proved its worth as a simple, inexpensive and reproducible method for the chemical and biological screening of plant extracts. It provides a basic idea of polarity of a particular chemical constituent.[44] Development of TLC plates with appropriate group reagents indicates the presence of anthraglycosides, arbutin, flavonoids, cardiac glycosides and coumarins in HP powder. The pattern of bands on TLC plates provides fundamental data and is used to demonstrate the consistency and stability of herbal components. It is a potent and rapid way to distinguish between chemical classes which may not be fulfilled by macroscopic and microscopic analysis.[45] TLC is most recommended technique to create the fingerprints of herbal medicines because of its simplicity, versatility, specific sensitivity and easy sample preparation.[46] Thus, TLC is a convenient method of determining the quality and possible adulteration of herbal

Table 4: Preliminary phytochemical analysis of different solvent extracts of HP

	HPHx	HPHp	HPBz	HPEa	HPCl	HPAc	HPBu	HPEt	HPMt	HPAq
Tannin	-	-	-	-	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	+	+	+	+	+
Amino acid	-	-	-	-	-	-	-	-	-	+
Resin	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	+	+	++	+	++	+	+	++	++
Flavonoids	+	+	++	++	-	+++	+	+	+	+++
Alkaloids	-	-	-	-	-	-	-	-	-	-

(+++) appreciable amount; (++) moderate; (+) trace amount; (-) completely absent.

Table 5: *In vitro* antioxidant potential and quantitative phytochemical analysis of HP through successive solvents extraction

	DPPH IC_{50} (mg/ml)	ABTS IC_{50} (mg/ml)	SO IC_{50} (mg/ml)	NO IC_{50} (mg/ml)	MC IC_{50} (mg/ml)	FRAP mg AAE/mg EW	TPC (mg Gallic Acid eq./mg EW)	TFC (mg quercetine eq./mg EW)	TOPC (mg catechol eq./ mg EW)
HPHx	2.156 ± 0.148^{d}	0.171 ± 0.003^{f}	NA	2.990 ± 0.145^{a}	0.976 ± 0.084^{a}	9.311 ± 0.030^{g}	94.353 ± 0.074^{h}	12.627 ± 0.042^{b}	18.983 ± 0.011^{c}
HPHp	0.653 ± 0.003^{ab}	0.089 ± 0.001^{c}	NA	NA	0.328 ± 0.036^{a}	2.994 ± 0.006^{c}	361.413 ± 0.00^{a}	NA	88.859 ± 0.018^{a}
HPBz	1.094 ± 0.256^{bc}	$0.091 \pm 0.012c^{d}$	7.147 ± 0.073^{e}	NA	a	4.955 ± 0.088^{e}	139.2 ± 0.082^{d}	15.459 ± 0.045^{a}	14.128 ± 0.004^{e}
HPEa	0.465 ± 0.1667^{a}	0.062 ± 0.001^{b}	1.197 ± 0.276^{b}	3.516 ± 0.379^{a}	NA	1.889 ± 0.084^{b}	176.077 ± 0.238^{c}	7.326 ± 0.033^{d}	19.021 ± 0.005^{c}
HPCl	1.511 ± 0.057^{c}	0.065 ± 0.003^{b}	3.379 ± 0.079^{c}	NA	1.272 ± 0.061^{a}	9.931 ± 0.011^{h}	100.849 ± 0.046^{g}	NA	33.443 ± 0.02^{b}
HPAc	0.256 ± 0.034^{a}	0.037 ± 0.004^{a}	0.782 ± 0.311^{ab}	5.046 ± 0.476^{b}	4.278 ± 0.193^{c}	1.064 ± 0.029^{a}	272.988 ± 0.2^{b}	15.387 ± 0.071^{a}	17.406 ± 0.044^{d}
HPBu	0.612 ± 0.023^{a}	0.106 ± 0.012^{d}	0.833 ± 0.178^{ab}	9.224 ± 0.010^{d}	NA	3.544 ± 0.038^{d}	111.389 ± 0.088^{f}	NA	13.628 ± 0.019^{f}
HPEt	3.288 ± 0.594^{e}	0.249 ± 0.002^{g}	4.482 ± 0.511^{d}	6.832 ± 0.0568^{c}	NA	14.001 ± 0.12^{i}	24.419 ± 0.085^{j}	1.126 ± 0.006^{f}	5.902 ± 0.007^{h}
HPMt	1.243 ± 0.148^{c}	0.094 ± 0.006^{cd}	12.331 ± 0.158^{f}	NA	NA	6.942 ± 0.482^{f}	39.162 ± 0.263^{i}	1.716 ± 0.032^{e}	3.742 ± 0.022^{i}
HPAq	0.412 ± 0.015^{a}	0.134 ± 0.012^{e}	0.369 ± 0.151^{a}	29.608 ± 0.416^{c}	3.10 ± 0.946^{b}	1.726 ± 0.028^{b}	122.434 ± 0.168^{e}	10.053 ± 0.112^{c}	13.556 ± 0.077^{g}

Values with different letters (a, b, c, d, e, f, g, h & i) are significantly ($p < 0.05$) different from each other by Duncan's multiple range test (DMRT); NA: Not applicableAQ.

products. Over the ages, ethnomedicines have played a pivotal role in preventing and treating numerous human diseases and disorders. The credit of this ability may be given to the antioxidant potential of these plants used as ethnomedicine. For supporting the use of HP in traditional healing system, *in vitro* antioxidant activity of the various solvent extracts of HP was investigated. The previous studies have mentioned that no single method is sufficient to determine the antioxidant activity in a sample.[47] Hence various free radical scavenging activities of different solvent extracts of HP was evaluated. In DPPH, $ABTS^{+}$ and FRAP, acetone extract showed better activity along with high amount of TPC and TFC. Similar findings were noticed in *Bergenia ciliata* leaves where the acetone extract had exhibited significant free-radical scavenging property when compared with other non polar and polar compounds.[48] Heptane extract exhibited highest TPC and TOPC as compared to other extracts while TFC was found to be highest in the benzene extract. Chelation of metal, SO and NO scavenging activity was more in HPHp, HPHx and HPAq respectively. From the overall result, it was observed that the efficacy of each extract varied against different free radicals in specific assays indicating the complexity of mechanisms and the diverse chemical nature of the active phytoconstituents present in plants.[49] Qualitative phytochemical studies on the different solvent extracts of HP revealed that some solvents are good for extraction of phytoconstituents while some others are not so suitable. It is given in Table 4 that triterpenoids was present only in HPAc, HPBu, HPEt, HPMt and HPAq. Amino acids were found to be extracted only by the aqueous extract. Alkaloid was absent which was evidenced by the absence of Dragendorff's band in TLC method. All the extracts indicated the presence of cardiac glycosides and flavonoids particularly extracted by acetone and aqueous solvents in large amount. It could be assumed that the presence of the above phytoconstituents in some selected plant extracts alone or in combination might be credited for the antioxidant potential found in HP. To determine this view, Pearson correlation test was done between the phytochemical contents and the antioxidant activity of HP. The results in Table 6 showed a significant positive correlation between FRAP with DPPH and $ABTS^{+}$ while FRAP showed significant negative correlation with TPC indicating that phenolic content of HP might be responsible for causing ferric reducing potential. But there was no correlation between TPC, TFC and TOPC with the other radical scavenging activities of HP. Our observation supports the study of antioxidants on *Fraxinus floribunda* bark where no correlation was obtained between phenolics and antioxidant activity.[50] Similar finding was also reported in a number of medicinal plant extracts in which no correlation was found between total phenol content and antioxidant capacity.[51] Therefore it might be possible that there were other non-phenolic phytochemicals such as amino acids, uronic acids, ascorbic acid,

Table 6: Pearson's correlation coefficients between phytochemical content and free radical scavenging activity of HP

	DPPH	ABTS+	SO	NO	MC	FRAP	TPC	TFC
ABTS$^+$	0.826**	-	-	-	-	-	-	-
SO	0.367	0.099	-	-	-	-	-	-
NO	-0.302	0.084	-0.359	-	-	-	-	-
MC	-0.613	-0.347	-0.852	0.231	-	-	-	-
FRAP	0.964**	0.700*	0.422	-0.308	-0.586	-	-	-
TPC	-0.616	-0.554	-0.558	-0.129	0.044	-0.660*	-	-
TFC	-0.496	-0.482	-0.423	0.000	-0.044	-0.548	0.741	-
TOPC	-0.244	-0.263	-0.461	-0.259	-0.528	-0.220	0.781**	0.771*

**Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

tocopherol, pigments etc which could be responsible for the antioxidant capacity.[52,53] The strong positive correlation between DPPH and ABTS$^+$ suggests that the same phytoconstituents might be causing these free radicals scavenging activity of HP. TPC, TFC and TOPC were also found to be strongly correlated with each other.

CONCLUSION

All the scientific investigations support the traditional use of the powdered polyherb, HP for the treatment of heart problems which might be due to the accumulation of the free radicals. The Pearson correlation analysis suggested that the antioxidant potential possessed by HP was not due to phenolic compounds but it might be credited to other non-phenolic compounds found in plants. Further studies should be carried out in future using the animal models to confirm its antioxidant potential and its *in vivo* effectiveness of ethno-medicinal use. The data of pharmacognostic studies could be useful as a reference for the authentication and accurate identification of the polyherbal formulation, HP and also to differentiate it from its adulterants and alternatives.

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CONFILCTS OF INTEREST

Authors declare no conflict of interest.

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